

CLAIMS

1. A 2-hydroxyisoflavanone dehydratase, substantially comprising a sequence of amino acids 1-328 represented by SEQ ID NO: 1.
2. A 2-hydroxyisoflavanone dehydratase according to claim 1, wherein a dehydration reaction is accelerated by acting on 2,7-dihydroxy-4'-methoxyisoflavanone or 2,5,7-trihydroxy-4'-methoxyisoflavanone to thereby generate formononetin or biochanin A.
3. A polynucleotide, substantially comprising:
 - a nucleotide sequence encoding the 2-hydroxyisoflavanone dehydratase according to claim 1 or 2; or
 - a nucleotide sequence complementary to the nucleotide sequence.
4. A polynucleotide, which encodes a 2-hydroxyisoflavanone dehydratase consisting of 1-1178 bases, and is represented by SEQ ID NO: 2.
5. A polynucleotide, having 50% or more homology to a nucleotide sequence included in SEQ ID NO: 2, and encoding a 2-hydroxyisoflavanone dehydratase.

6. A polynucleotide according to any one of claims 3 to 5, which is obtained by cloning from *Glycyrrhiza echinata*.

7. A polynucleotide, which hybridizes at least part of a polynucleotide having a nucleotide sequence of SEQ ID NO: 2 or a nucleotide sequence complementary to the nucleotide sequence.

8. A polynucleotide, which can function as a primer or a probe for a nucleotide sequence encoding a 2-hydroxyisoflavanone dehydratase or cDNA of the 2-hydroxyisoflavanone dehydratase, which can be hybridized with a successive sequence of at least 15 of SEQ ID NO: 2 or a polynucleotide complementary to the successive sequence.

9. A 2-hydroxyisoflavanone dehydratase, encoded by the polynucleotide according to any one of claims 3 to 6.

10. A method of dehydrating a 2-hydroxyisoflavanone comprising using a protein encoded by the polynucleotide according to any one of claims 3 to 6.

11. A method of producing an isoflavonoid comprising using at least flavanone, 2-hydroxyisoflavanone synthase (IFS), and a protein encoded by the polynucleotide according to any one of claims 3 to 6.

12. A vector, comprising the polynucleotide according to any one of claims 3 to 6 inserted therein.

13. A recombinant DNA or RNA, comprising an expression system from which the polynucleotide according to any one of claims 3 to 6 can be expressed in a host cell.

14. A host cell transformed by the vector according to claim 12.

15. A transformed host cell according to claim 14, wherein the host cell comprises yeast.

16. A host cell according to claim 14, wherein the host cell comprises a recombinant *E. coli* cell of Accession No: FERM BP-08662.

17. A method of manufacturing 2-hydroxyisoflavanone dehydratase, comprising incubating the host cell according to any one of claims 14 to 16.

18. A method of producing isoflavonoid comprising using the host cell according to any one of claims 14 to 16.

19. A method of producing isoflavonoid comprising using a host cell

transformed by the polynucleotide according to any one of claims 3 to 6 and a polynucleotide encoding a 2-hydroxyisoflavanone synthase (IFS).

20. A transgenic plant, comprising the polynucleotide according to any one of claims 3 to 6 introduced therein.

21. A transgenic plant according to claim 20, wherein the transgenic plant comprises a leguminous plant.

22. A method of producing isoflavonoid comprising using the plant according to claim 20 or 21.

23. A method of modifying isoflavonoid comprising using the plant according to claim 20 or 21.

24. A 2-hydroxyisoflavanone dehydratase, substantially comprising a sequence of amino acids 1-319 represented by SEQ ID NO: 3.

25. A 2-hydroxyisoflavanone dehydratase according to claim 24, wherein a dehydration reaction is accelerated by acting on 2,7,4'-trihydroxyisoflavanone or 2,5,7,4'-tetrahydroxyisoflavanone to thereby generate daidzein or

genistein.

26. A polynucleotide, substantially comprising:

a nucleotide sequence encoding the 2-hydroxyisoflavanone dehydratase according to claim 24 or 25; or

a nucleotide sequence complementary to the nucleotide sequence.

27. A polynucleotide, which encodes a 2-hydroxyisoflavanone dehydratase consisting of 1-960 bases, and is represented by SEQ ID NO: 4.

28. A polynucleotide, having 50% or more homology to a nucleotide sequence included in SEQ ID NO: 4, and encoding a 2-hydroxyisoflavanone dehydratase.

29. A polynucleotide according to any one of claims 26 to 28, which is obtained by cloning from soybeans.

30. A polynucleotide, which hybridizes at least part of a polynucleotide having a nucleotide sequence of SEQ ID NO: 4 or a nucleotide sequence complementary to the nucleotide sequence.

31. A polynucleotide, which can function as a primer or a probe

for a nucleotide sequence encoding a 2-hydroxyisoflavanone dehydratase or cDNA of the 2-hydroxyisoflavanone dehydratase, which can be hybridized with a successive sequence of at least 15 of SEQ IDNO: 4 or a polynucleotide complementary to the successive sequence.

32. A 2-hydroxyisoflavanone dehydratase, encoded by the polynucleotide according to any one of claims 26 to 29.

33. A method of dehydrating a 2-hydroxyisoflavanone comprising using a protein encoded by the polynucleotide according to any one of claims 26 to 29.

34. A method of producing an isoflavonoid comprising using at least flavanone, 2-hydroxyisoflavanone synthase (IFS), and a protein encoded by the polynucleotide according to any one of claims 26 to 29.

35. A vector, comprising the polynucleotide according to any one of claims 26 to 29 inserted therein.

36. A recombinant DNA or RNA, comprising an expression system from which the polynucleotide according to any one of claims 26 to 29 can be expressed in a host cell.

37. A host cell transformed by the vector according to claim 35.

38. A transformed host cell according to claim 37, wherein the host cell comprises yeast.

39. A host cell according to claim 37, wherein the host cell comprises a recombinant *E. coli* cell of Accession No: FERM BP-08661.

40. A host cell transformed by a vector where a polypeptide encoding a 2-hydroxyisoflavanone synthase (IFS) is inserted and a vector where the polynucleotide according to any one of claims 26 to 29 is inserted.

41. A transformed host cell according to claim 40, wherein the host cell comprises yeast.

42. A host cell according to claim 41, wherein the host cell comprises a recombinant yeast *E. coli* cell of Accession No: FERM BP-08663.

43. A method of manufacturing 2-hydroxyisoflavanone dehydratase, comprising incubating the host cell according to any one of claims 37 to 42.

44. A method of producing isoflavonoid comprising using the host

cell according to any one of claims 37 to 42.

45. A transgenic plant, comprising the polynucleotide according to any one of claims 26 to 29 introduced therein.

46. A transgenic plant according to claim 45, wherein the transgenic plant comprises a leguminous plant.

47. A method of producing isoflavonoid comprising using the plant according to claim 45 or 46.

48. A method of modifying isoflavonoid comprising using the plant according to claim 45 or 46.

49. A polynucleotide, encoding an enzyme having a motif of carboxylesterase and catalyzing a dehydration reaction.

50. A polynucleotide, encoding an enzyme having a motif of carboxylesterase and catalyzing a dehydration reaction of a 2-hydroxyisoflavanone.